Signal transduction

***ILOs*: by the end of this chapter, you will be able to:**

1. Compare between different types of hormones.
2. Explain the mechanism of action of lipophilic hormones.
3. Explain mechanism of action of hormones using cAMP as second messenger.
4. Correlate disruption in hormone signaling to clinical disorders
5. Interpret role of calcium as a mediator of hormone action.
6. Explain mechanism of action of hormones using cGMP as second messenger.
7. Discuss mechanism of action of hormones acting on tyrosine kinases.

OVERVIEW

- Integration of the function and responses of different body organs and tissues requires communication that is carried out by **chemical messengers** which travel from one cell to another, or by direct contact of cells with the extracellular matrix or with each other.
- **A hormone:** is a signaling molecule produced by glands in multicellular organisms that are transported by the circulatory system to target distant organs to regulate physiology and behavior.
- When a chemical messenger binds to a receptor the signal it is carrying must be converted into an intracellular response. This conversion is called **signal transduction**.

What is a signal?

It represents information carried by the chemical messenger and detected by its specific receptors.

What is signal transduction?

It is the conversion of extracellular information (signal), detected by the specific receptor, into intracellular chemical change.

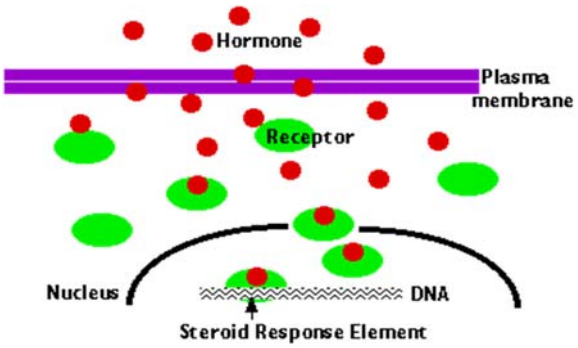
CELLULAR RECEPTORS

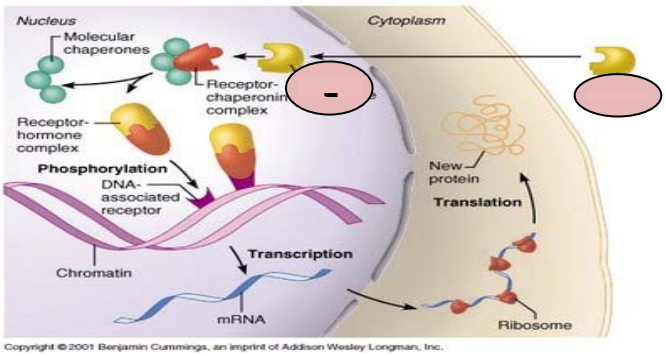
I- Definition: receptors are cell-associated recognition molecules of high degree of discrimination which enable the cell to distinguish and bind to specific hormones present at a very low concentration in the extracellular fluid.

II- Types of receptors:

(A)- Intracellular receptors

- These receptors bind lipophilic hormones after their diffusion into the cell:
- **Nuclear receptors** that are located in the nucleus e.g thyroid hormones.
- **Cytosolic receptors** that are present in the cytosol e.g steroid hormones.





(B)- Cell membrane receptors

- These receptors are located on the plasma membrane of target cells. They bind hydrophilic hormones that cannot traverse the cell membrane (lipid bilayer). This type of receptors is further subdivided into:

1- Serpentine receptors (G-protein coupled receptors):

- Using adenyl cyclase as effector ↑ cAMP e.g. β-adrenergic receptor.
- Using phospholipase C as effector ↑ IP3, Ca, DAG e.g. α1- adrenergic receptor.

2- Receptors with intrinsic enzymatic activity:

- With guanyl cyclase activity: e.g. ANP and NO receptors.
- With tyrosine kinase activity: e.g. insulin and growth factor receptors.

3- Receptors that lack enzymatic activity but attract and **activate cytoplasmic kinases** (JAKS) e.g. JAK-STAT cascade of growth hormone.

4- Ion channel receptors: signal transduction consists of the conformational change when ligand binds. Most small molecule neurotransmitters and some neuropeptides use ion channel receptors.

CLASSIFICATION OF HORMONES

Classification according to solubility & mechanism of action :

1- Hydrophilic hormones: water soluble.

2- Lipophilic hormones: insoluble in water.

Comparison between lipophilic and hydrophilic hormones:

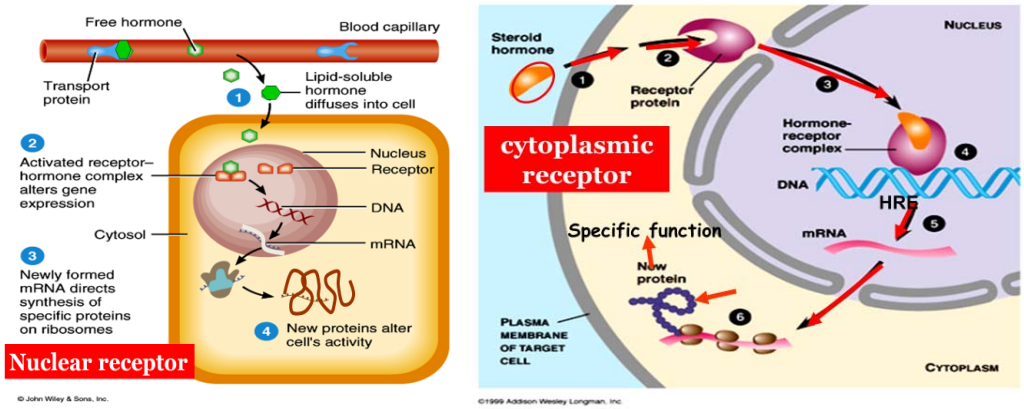
	Lipophilic group	**Hydrophilic group**
Types	Thyroid hormones, Steroid hormones: Glucocorticoids, Mineralocorticoids, Sex hormones. Active vitamin D, Retinoids and Eicosanoids.	Peptides & Protein hormones. e.g. Insulin, Glucagon, GH Amino acid-derived Hormones e.g. Catecholamines, e.g. Serotonin, Melatonin
Solubility	Fat soluble.	Water soluble.
Transporter proteins	present	absent
Plasma half life	Long (hours to days)	Short (minutes)
Receptors	Intracellular	Plasma membrane-associated
Mediator	Receptor-hormone complex	cAMP, cGMP, calcium, IP3, DAG, Kinase cascade, Transcription proteins (STAT)

A- Lipophilic hormones:

- After secretion, these hormones associate with plasma transport (carrier) proteins as they are insoluble in the aqueous medium of plasma. This prolongs the plasma half-life of the hormone.
- The free hormone, which is the biologically active form, readily **traverses** the lipophilic plasma membrane of all cells and binds receptors in either the cytosol or nucleus of target cells.
- In the cells they bind to their intracellular receptors forming **hormone-receptor complex**. These receptors can be located in the cytoplasm (e.g. glucocorticoids & aldosterone) or in the nucleus (e.g thyroid hormones, calcitriol, estrogen, progesterone) of target cells.
- Hormone binding to its receptor triggers changes in the conformation of receptor protein so it becomes capable of binding with high affinity to a specific DNA sequence called the **hormone response element** (**HRE**). This

binding can either enhance or suppress the **expression** of specific genes adjacent to HRE.

- So, RNA synthesis and subsequent protein synthesis is changed inducing the intracellular metabolic effect of the hormone (hours and days are required for these changes).

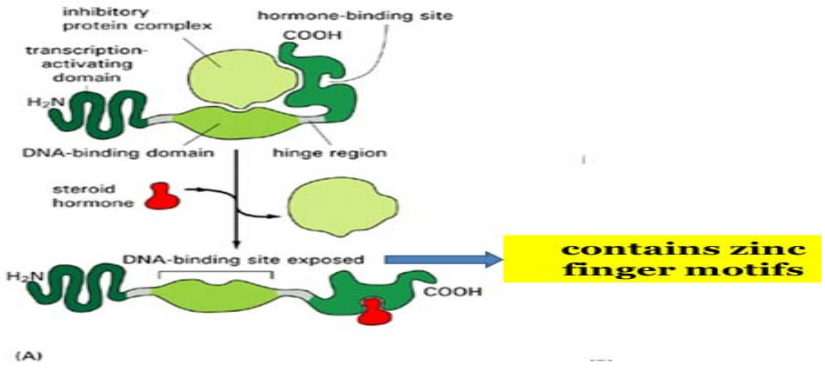


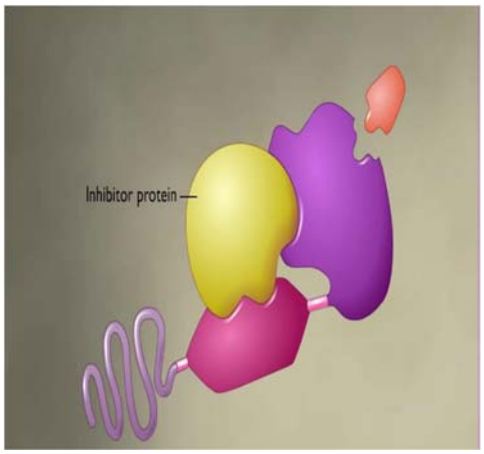
- Intracellular receptors of this group are structurally characterized by presence of 3 domains:

1-A carboxyl terminal region that binds the hormone.

2- A central DNA binding domain.

3- An amino terminal acting as gene enhancer or gene suppressor.





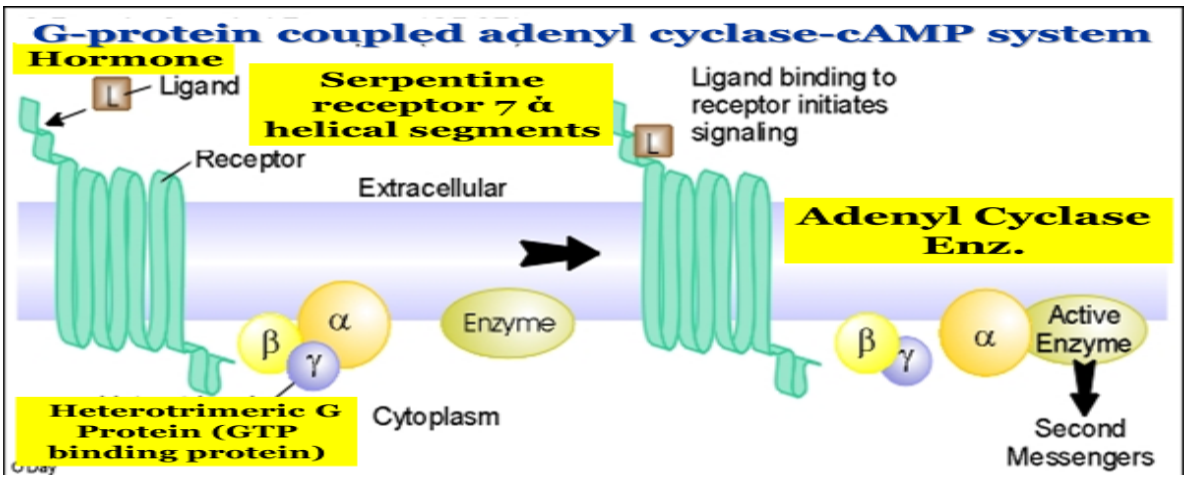
B- Hydrophilic hormones:

- They are water-soluble hormones that have no transport proteins and have a short plasma half-life. They initiate a response by binding to a receptor located in the plasma membrane of target cells.
- They communicate with intracellular metabolic processes through intermediary molecules called **second messengers** (the hormone itself is the first messenger), which are generated as a consequence of the hormone-receptor interaction.
- The mechanism of action of group 2 hormones can best be discussed in terms of the intracellular messenger they generate:
  1- cAMP
  2- Ca,
  3- cGMP
  4- kinases

1- <u>cAMP as a second messenger:</u>

Hormones acting by this method: **Glucagon**, **β adrenergic catecholamines**, FSH, LH, ACTH, TSH.

- Epinephrine binds to its plasma membrane heptahelical or **serpentine** receptor (with hydrophobic regions that "snake" back and forth across the plasma membrane seven times).
- Hormone-receptor binding promotes a conformational change in the receptor's intracellular domain that affects its interaction with the heterotrimeric (αβγ) guanosine nucleotide-binding protein (**G protein**).
- **<u>The G protein</u>**, stimulated by the activated receptor, exchanges bound GDP for GTP on α subunit with concomitant dissociation of βγ from α.
- The active G-protein dissociates from the occupied receptor and the GTP-loaded Gα binds to the effector enzyme, adenyl cyclase, activating it. This stimulatory G-protein is termed Gs.
- At least 15 different α subunits are known.
- Gα subunits are distinguished from each other by subscripts including s, i, and q (Gαs, Gαi, and Gαq), Gs stimulates adenylate cyclase enzyme while Gi inhibits it. Gq stimulates phospholipase C.



- Adenyl cyclase (AC) is an integral protein of the plasma membrane, with its active site on the cytosolic face. It catalyzes the synthesis of cAMP from ATP.
- Cyclic AMP binds to **protein kinase A** (PKA) (cAMP-dependent protein kinase) and allosterically activates it. The inactive form of PKA contains two catalytic subunits (C) and two regulatory subunits (R).
- The tetrameric R2C2 complex is catalytically inactive, because each R subunit occupies the substrate-binding site of one C subunit.
- When cAMP binds to two sites on each R subunit, the R subunits undergo a conformational change and the R2CA complex dissociates to yield two free (C) subunits.
- These catalytic subunits are able to phosphorylate target proteins and enzymes (on serine or threonine residues) to produce cellular effects.
- **Effect of cAMP on transcription**: The effect of cAMP on transcription is mediated by CREB protein (cAMP response element-binding protein): when CREB protein is phosphorylated by PKA, it binds the coactivator CBP (CREB-binding protein) and becomes a highly potent transcription factor.
- **This Hormonal action is terminated:**
  1- Stimulation of AC by Gα is self-limiting; Gα has an inherent **GTPase activity** that hydrolyzes bound GTP to GDP. The now inactive Gα dissociates from adenyl cyclase and re-associates with the β γ subunits, to be ready for another cycle of activation.
  2- Cyclic AMP is short-lived. It is quickly degraded by **phosphodiesterase** (**PDE**) to 5-AMP, which is not active as a second messenger. The intracellular signal therefore persists only as long as the hormone receptor remains occupied by epinephrine.
  3- **Phosphatases** remove phosphate from phosphorylated proteins and thus terminate the hormonal action.

- **Examples of hormone which stimulate adenyl cyclase and increase cAMP** levels are: β-adrenergic catecholamine, glucagon, ACTH, TSH, FSH, LH.
- **Examples of hormone which inhibit adenyl cyclase and lower cAMP** levels, and suppressing protein phosphorylation are: somatostatin and α2-adrenergic catecholamine. Their binding to their receptors leads to activation of an inhibitory G protein (Gi), structurally homologous to Gs, that inhibits adenyl cyclase and lowers cAMP. The alpha subunit in Gi, (αi) differs from that in Gs (αs) but β and γ are the same in both.

Disruption of G-Protein signaling causes disease

- Cholera toxin, an enzyme secreted by *vibrio cholerae* found in contaminated drinking water, catalyzes the transfer of ADP-ribose from NAD to the α-subunit of Gs, blocking its GTPase activity and thereby rendering Gs permanently activated.
- This results in continuous activation of the adenyl cyclase of intestinal epithelial cells and chronically high cAMP, which triggers constant secretion of Cl, HCO3 and water into the intestinal lumen resulting in dehydration and electrolyte loss.
- Pertussis toxin, an enzyme produced by *Bordetella pertussis*, catalyzes ADP ribosylation of Gi, preventing displacement of GDP by GTP and blocking inhibition of adenyl cyclase by Gi. This defect produces 2 of whooping cough symptoms.

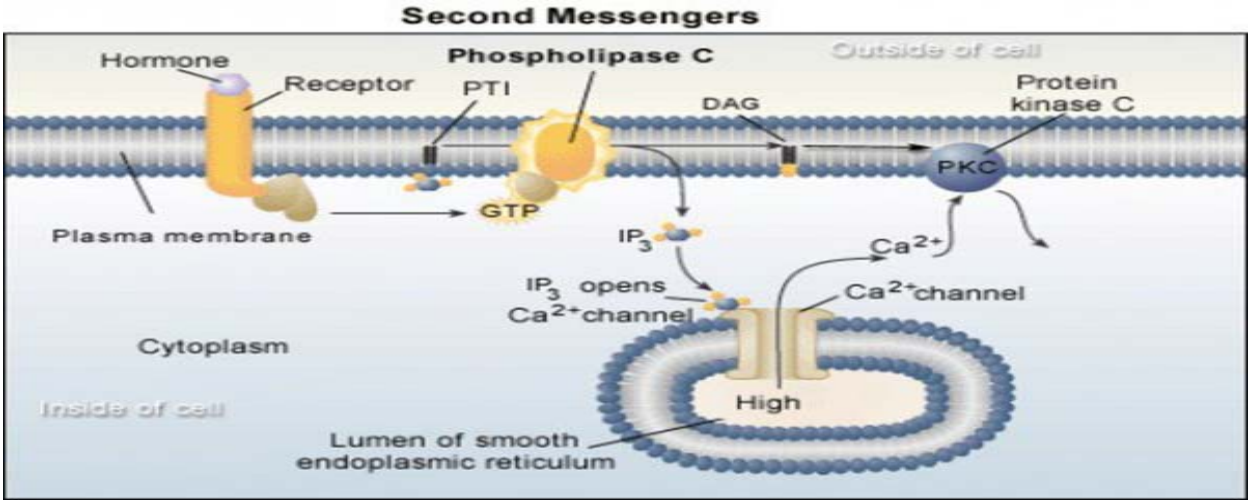
PROTEIN Gq AND PHOSPHOLIPASE C

2- Calcium and/or phosphatidyl Inositol phosphate as second messengers:

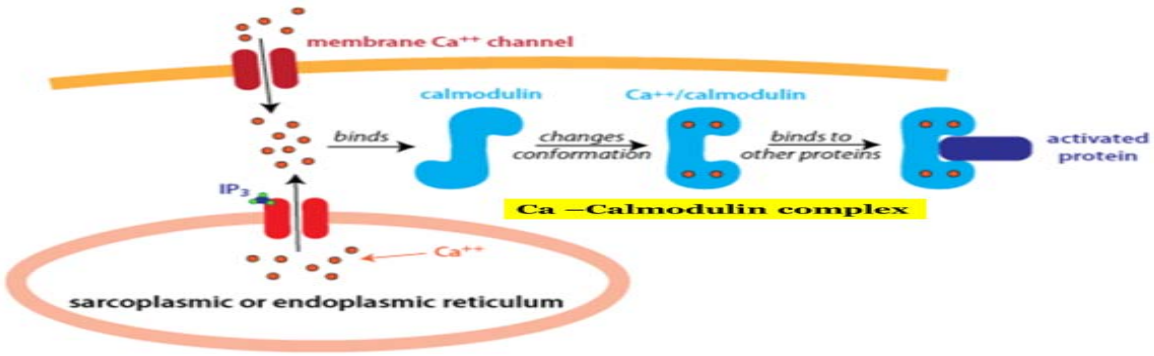
- Normally, **cytosolic Ca** is kept very **low** by the action of Ca pumps in the ER, mitochondria, and plasma membrane. Hormonal, neural, or other stimuli cause either an influx of Ca into the cell through specific Ca channels in the plasma membrane or the release of sequestered Ca from the ER or mitochondria, in either case raising the cytosolic Ca will trigger a cellular response.
- when a hormone of this class binds its specific serpentine receptor in the plasma membrane, the **receptor-hormone complex** catalyzes GTP-GDP exchange on a unique G protein, **Gq**, activating it exactly as the β-adrenergic receptor activates Gs.
- The α-subunit of activated Gq dissociates from βγ and **activates PLC** (phospholipase C), a membrane-bound enzyme, which hydrolyzes phosphatidylinositol 4,5-bisphosphate **(PIP2)** in the plasma membrane

into **DAG** (diacylglycerol) and **IP3** (inositol 1,4,5-triphosphate) which act as **second messengers**.

- Inositol triphosphate **IP3**, a water-soluble compound, diffuses from the plasma membrane to the endoplasmic reticulum (**ER**), where it binds to IP3-gated **calcium channel receptors** within the ER membrane and causes them to open. Sequesterd Ca is thus released into the cytosol, and the **cytosolic Ca rises** sharply.
- One effect of elevated Ca is the activation of **PKC** (protein kinase C or Ca-dependent protein kinase). **DAG** is hydrophobic and so it remains in the membrane and cooperates with Ca in **activating PKC**.
- PKC phosphorylates Ser or Threonine residues of specific target proteins, changing their catalytic activates.



- The elevated intracellular Ca binds and activates specific cellular proteins either directly or through **calmodulin protein**.
- **Calmodulin** is an integral regulatory subunit of a family Ca\calmodulin-dependent protein kinases and associates with a variety of proteins. It has **4 binding sites for Ca.**
- When Ca occupies its 4 binding sites, calmodulin exhibits a conformational change that activates the **kinases**. The kinases then phosphorylate a number of target enzymes, modifying their activities.



- ***N.B.*** *Phorbol esters are synthetic tumor promoters that are potent activators of PKC. They mimic cellular DAG but they are not rapidly metabolized. By continuously activating PKC, they interfere with the normal regulation of cell growth and division.*

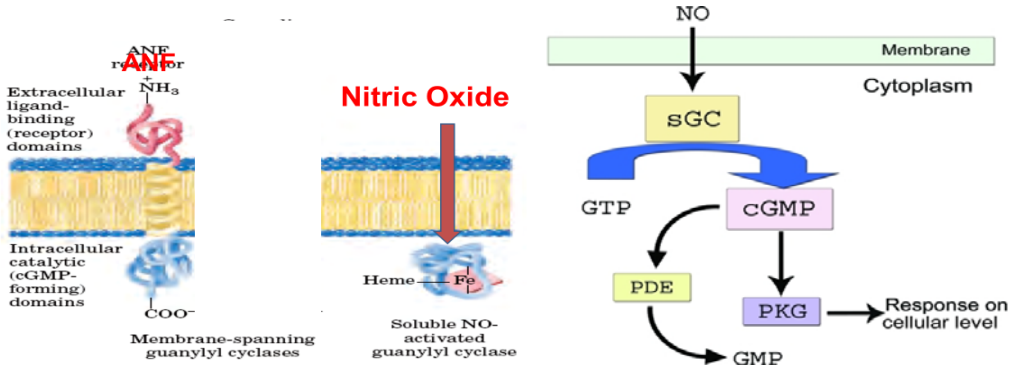
3- cGMP as a second messenger:

- **Guanylyl cyclase** is a type of receptor enzymes. When activated, it produces guanosine 3,5-cyclic monophosphate (cGMP) from GTP.

GTP **Guanylyl cyclase** cGMP

a- Membrane spanning guanylyl cyclase: that is activated by its extracellular ligand: Atrial natriuretic factor (**ANF**).

b- A soluble cytosolic guanylyl cyclase: with a tightly associated heme group that is activated by nitric oxide (NO).



4- The second messenger is a kinase:

- Signal transduction is carried out by tyrosine kinases which autophosphorylates tyrosine residues in the receptor and in specific target proteins. The **Tyrosine kinase** activity responsible to produce phosphotyrosines may belong to the receptor itself or to a Tyr kinase that associates with the receptor.

- A **phosphatase** can disrupt the signal by removing phosphate or the dephosphorylated protein may be the signal.

A- Signal transduction through receptors with intrinsic tyrosine kinase activity:

- The **tyrosine kinase** receptors generally exist in the membrane as **monomers** with a single membrane-spanning helix.
- One molecule of the **growth factor** generally binds two molecules of the receptor and promotes their **dimerization**.
- Once the receptor dimer has formed, the intracellular tyrosine kinase domains of the receptor phosphorylate each other on certain tyrosine residues (**autophosphorylation**).
- The phosphotyrosine residues form specific binding sites for **signal transducer proteins**.

The insulin receptor:

- The insulin receptor is a member of the **tyrosine kinase** family of receptors. Unlike other growth factor receptors, the insulin receptor exists in the membrane as a **dimer**, with each half containing an **α** and a **β** subunit.
- The insulin receptor is a **heterotetramer** glycoprotein consisting of two identical α subunits protruding from the outer face of the plasma membrane and two transmembrane β-subunits with their carboxyl termini protruding into the cytosol.
- The **α-subunits** contain the **insulin binding domain**, and the intracellular domains of the **β-subunits** contain the **tyrosine kinase activity**.

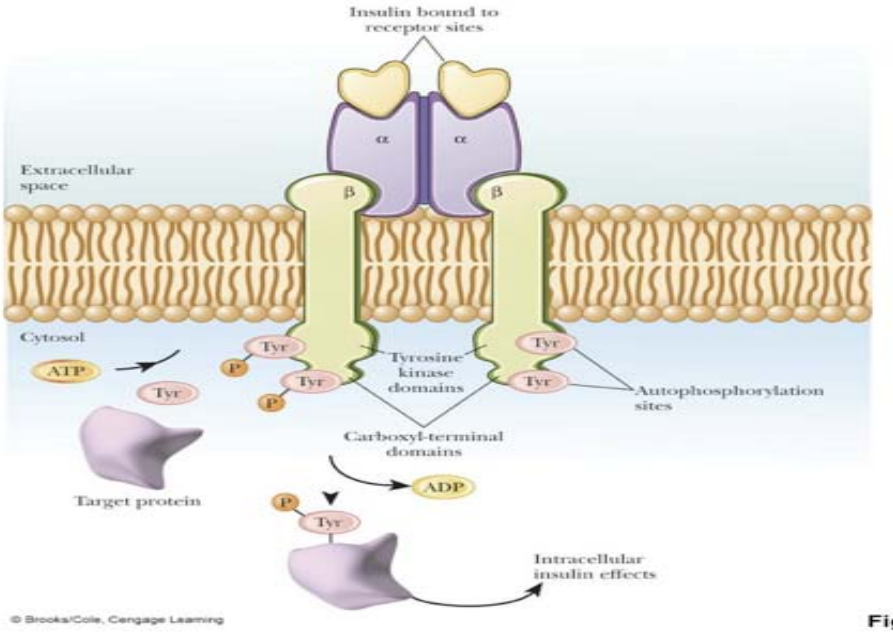
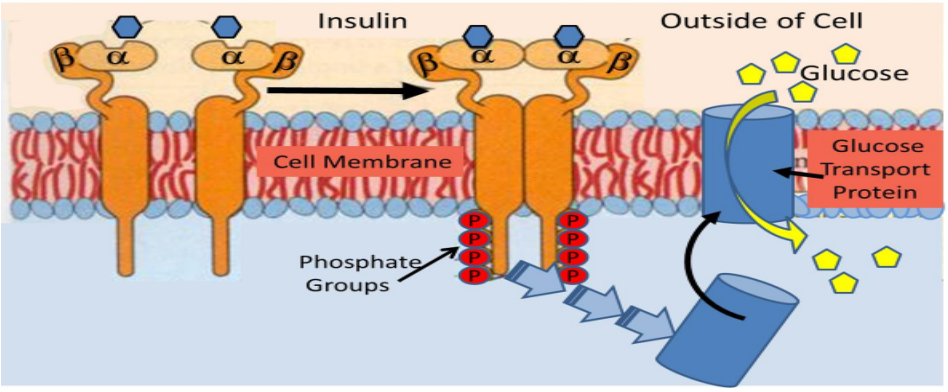


Fig. 24-17, p. 729

- Signaling begins when insulin binding activates receptor ***tyrosine kinase*** activity in the intracellular domain of the β subunit.
- Tyrosine residues of the β subunit are **autophosphorylated**. This auto-cross-phosphorylation opens the active site so that the enzyme can phosphorylate Tyr residues of other target proteins.
- Receptor tyrosine kinase phosphorylates other proteins, for example, insulin receptor substrates (**IRS**).
- Phosphorylated IRS promotes activation of other protein **kinases** and **phosphatases**, leading to biologic actions of insulin.

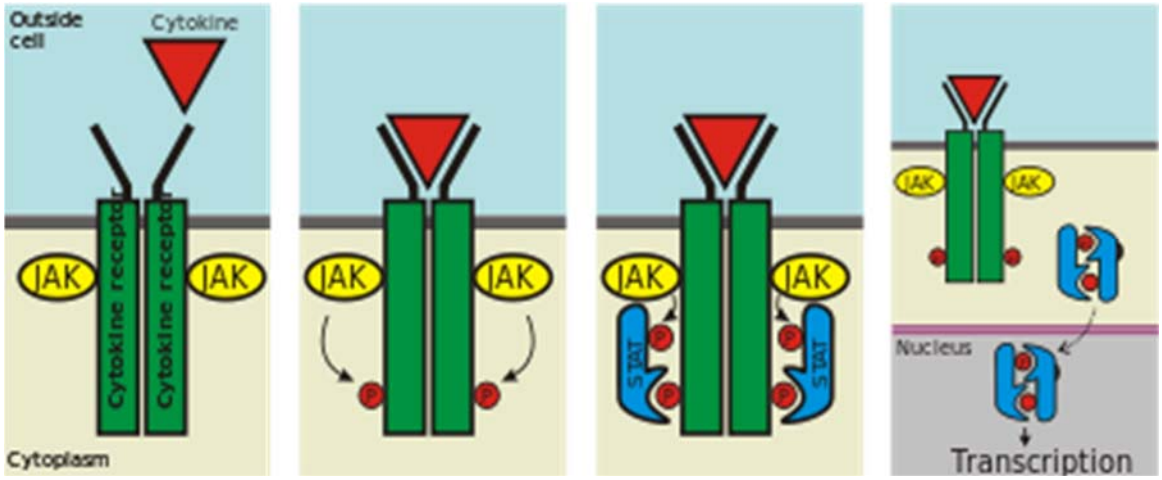


- In this way, the receptor activates several enzyme cascades which produce the metabolic effects of insulin.
- Rapid effects as dephosphorylation of enzymes and increasing number of GLUT-4 in the membrane of muscle and adipose tissue and
- Delayed effects controlling gene expression.

B- Receptors with no intrinsic enzyme activity but use cytoplasmic tyrosine kinase activity (JAK-STAT receptors):

- Each receptor monomer has an **extracellular** domain, a **membrane-spanning** region, and an **intracellular** domain.
- Their signal transducer proteins, called **STAT's** (signal transducer and activator of transcription), are themselves gene-specific **transcription factors**. Thus, JAK-STAT receptors have a more direct route for propagation of the signal to the **nucleus** than tyrosine kinase receptors.
- N.B. *jak is an acronym for janus kinase (It was named for janus, a two-headed god of the romans). It has been suggested that it stands for " just another kinase".*
- As the cytokine binds to these receptors, they form **dimers** and may cluster. The receptor is activated and can now bind a **soluble tyrosine kinase** called **JAK** (janus kinase) and **activates** it.
- Active JAKs **phosphorylate** each other and tyrosine residues on the receptor, forming phosphotyrosine-binding sites for the STATs.

- **STATs** are inactive in the cytoplasm until they bind to the receptor complex, where they are also **phosphorylated** by the bound JAK.
- Phosphorylation changes the conformation of the **STAT**, causing it to dissociate from the receptor and **dimerize** with another phosphorylated STAT, thereby an activated **transcription factor**.
- The STAT dimer translocates to the nucleus and binds to a hormone response element (**HRE**) on DNA, thereby regulating gene transcription.
- Receptors for **cytokines** (**interleukins** and **interferons**) and for some hormones (such as **prolactin** and **growth hormone**) are examples.



Summary of Signal Transduction by Water-Soluble Hormones:

Pathway	G Protein	Enzyme	Second Messenger(s)	Protein Kinase	Examples
cAMP	G_s (G_i)	Adenyl cyclase	cAMP	Protein kinase A	Glucagon Epinephrine (β, α-2) Vasopressin (V2, ADH) kidney
PIP_2	G_q	Phospholipase C	DAG, IP_3, Ca^{2+}	Protein kinase C	Vasopressin (V1, V3) vascular smooth muscle Epinephrine (α_1)
cGMP	None	Guanyl cyclase	cGMP	Protein kinase G	Atrial natriuretic factor (ANF) Nitric oxide (NO)
Insulin, growth factors	Monomeric $p21^{ras}$	—	—	Tyrosine kinase activity of receptor	Insulin Insulin-like growth factor (IGF) Platelet-derived growth factor (PDGF) Epidermal growth factor (EGF)